Biomass and toxicity responses of poison ivy (*Toxicodendron radicans*) to elevated atmospheric CO_2

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Contributed by William H. Schlesinger, April 22, 2006

Contact with poison ivy (*Toxicodendron radicans*) is one of the most widely reported ailments at poison centers in the United States, and this plant has been introduced throughout the world, where it occurs with other allergenic members of the cashew family (Anacardiaceae). Approximately 80% of humans develop dermatitis upon exposure to the carbon-based active compound, urushiol. It is not known how poison ivy might respond to increasing concentrations of atmospheric carbon dioxide (CO_2), but previous work done in controlled growth chambers shows that other vines exhibit large growth enhancement from elevated CO_2. Rising CO_2 is potentially responsible for the increased vine abundance that is inhibiting forest regeneration and increasing tree mortality around the world. In this 6-year study at the Duke University Free-Air CO_2 Enrichment experiment, we show that elevated atmospheric CO_2 in an intact forest ecosystem increases photosynthesis, water use efficiency, growth, and population biomass of poison ivy. The CO_2 growth stimulation exceeds that of most other woody species. Furthermore, high-CO_2 plants produce a more allergenic form of urushiol. Our results indicate that *Toxicodendron* taxa will become more abundant and more "toxic" in the future, potentially affecting global forest dynamics and human health.

global change | forest ecology | *Rhus radicans*

Poison ivy [*Toxicodendron radicans* (L.) Kuntze] ranks among the most medically problematic plants in the United States (1, 2), annually causing >350,000 reported cases of human contact dermatitis (3). Its active component, urushiol, could be used for simulating the transmittal and subsequent symptoms of chemical warfare agents for the U.S. military (4). *T. radicans* is widely distributed and abundant in North America and also occurs in Central America, parts of Asia, Bermuda, and the Bahama Islands (5). It has been introduced in Europe (6, 7) and South Africa (8) and also in Australia and New Zealand, where it has become invasive and caused reported cases of contact dermatitis (9). Other allergenic *Toxicodendron* species occur in much of the world (10–12). Consequently, the response of *Toxicodendron* to global environmental change, particularly the current increase in global atmospheric carbon dioxide (CO_2) concentrations, bears consequences for human health on a panoptic scale.

Although the response of poison ivy to changing CO_2 has not been investigated previously, various vine species show large photosynthetic and growth increases with CO_2 enrichment when grown in noncompetitive conditions in enclosed, indoor growth chambers with optimal resource levels (13–15) and in low-light chambers simulating forest understory environments (16). In the first year of a 2-year field study in Tennessee, an exotic vine species (*Lonicera japonica*) grew significantly faster at elevated CO_2 (17). Stimulation of biomass production likely results from a positive feedback of high CO_2 for vines: With an increase in CO_2 concentration and a corresponding increase in photosynthesis, vines can allocate more photosynthate to additional photosynthetic tissue, because of a low allocation to support tissue relative to other woody growth forms (13, 14, 18, 19). Increasing abundance of woody vines is causing increased tree mortality and reduced tree regeneration in forests around the globe (18, 20–23), potentially resulting in shifts in community composition that may impact carbon cycling and biodiversity (23). Although it is unclear how elevated CO_2 will affect the growth of vines in forest environments, the contemporary increase in woody vine abundance may be the result of rising atmospheric CO_2 concentrations (19, 23).

When grown under low resource levels and/or in competitive environments, plants often show small growth enhancements from increased concentrations of CO_2 (24, 25). In competitive environments such as forest understories, plant growth may be limited by noncarbon resources such as soil moisture and nutrients. In such cases, additional photosynthate produced under elevated CO_2 may be allocated to carbon sinks, such as the generation of secondary carbon-based compounds (26). Thus, production of urushiol, the 3-*n* pentadecylcatechol hydrocarbon whose reaction with the human immune system is responsible for *Toxicodendron* dermatitis (27), may increase under elevated CO_2.

In this 6-year study at the Duke University Free-Air CO_2 Enrichment (FACE) experiment, we assessed the impacts of elevated atmospheric CO_2 (200 μl/liter above the ambient level of $\approx$370 μl/liter and representing the predicted global concentration at the middle of this century; ref. 28) on *in situ* growth and survivorship of poison ivy in an intact forest environment. Additionally, we determined effects of increased CO_2 on photosynthesis, water use, and production of five variants of the secondary compound, urushiol. The human dermatitis response to poison ivy is correlated with the ratio of [unsaturated:saturated] urushiol congeners (29, 30); the higher the relative unsaturated component, the more "poisonous" the plant is to humans.

Results and Discussion

Here we show that CO_2 enrichment increased *T. radicans* photosynthesis by 77% ($P < 0.01$), and increased the efficiency of plant water usage by 51% by reducing stomatal conductance ($P < 0.05$;

Conflict of interest statement: No conflicts declared.

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Abbreviation: FACE, Free-Air CO_2 Enrichment.

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Table 1. Physiological measurements on poison ivy (*Toxicodendron radicans*) leaves

	Light saturated photosynthesis, μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$	Stomatal conductance, mol $H_2O \cdot m^{-2} \cdot s^{-1}$	Apparent quantum yield, μmol $CO_2 \cdot \mu$mol·photons^{-1}	Light compensation point, μmol photons$\cdot m^{-2} \cdot s^{-1}$	Carboxylation rate, μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$	RuBP regeneration rate, μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$
Ambient CO_2	4.16 (0.23)	0.056 (0.005)	0.052 (0.004)	10.2 (2.0)	29.25 (2.33)	59.57 (3.51)
Elevated CO_2	7.38 (0.38)	0.037 (0.002)	0.051 (0.002)	10.3 (0.6)	28.49 (1.40)	57.96 (3.40)
E/A	1.77**	0.66*	0.98	1.01	0.97	0.97

Means are presented for foliage grown under ambient and elevated CO_2 concentrations, with SE in parentheses. E/A refers to the quotient of the elevated CO_2 data divided by the ambient CO_2 data. Carboxylation rate refers to the mean rate of Rubisco (RuBP) carboxylation. *, $P < 0.05$; **, $P < 0.01$ in two-tailed Student's *t* tests.

Table 1). Elevated CO_2 also stimulated the growth of poison ivy during the five growing seasons at the Duke FACE experiment (Fig. 1*A*). Although the average plant biomass in 1999 did not differ significantly between treatments, poison ivy grew faster with CO_2 enrichment ($P = 0.022$ from repeated-measures analysis) so that by 2004, ambient plants averaged 5.0 ± 0.6 g of dry weight and elevated CO_2 plants averaged 8.1 ± 0.8 g ($P = 0.008$), representing an average annual growth increase of 149% in elevated compared to ambient plants. This increase is notably larger than the 31% average increase in biomass observed for woody plants grown at two times the ambient CO_2 concentration under controlled conditions (31) (compared to 1.5 times ambient CO_2 at the Duke FACE experiment), and the ≈21% stimulation of overall net primary productivity in the first 5 years of the Duke FACE experiment (32).

CO_2 enrichment did not decrease light compensation points, increase quantum use efficiency, or affect the relationship of electron transport to Rubisco activity of *T. radicans* (Table 1). Thus, CO_2 did not affect the light-use efficiency of poison ivy plants in the forest understory. Although CO_2 is not significantly affecting aboveground stem survivorship of this species (Fig. 1*B*), by the end of the experiment, 35 ± 0.10% of the original stems (ramets) remained alive in the ambient plots, whereas 45 ± 0.09% remained alive in the high-CO_2 plots.

Overall, total population biomass, an integrator of plant growth, survivorship, and recruitment increased over time with CO_2 enrichment ($P = 0.046$; Fig. 2). After standardizing by initial 1999 biomass, the elevated-CO_2 population in 2004 had grown 150% larger, twice the 75% increase exhibited by the ambient-CO_2 population at the end of the study ($P = 0.005$). Further, the concentration of the unsaturated triene congener of urushiol increased 153% under elevated CO_2 ($P < 0.001$), whereas the saturated urushiol variant decreased by 61% ($P < 0.001$; Fig. 3), resulting in an increased [unsaturated:saturated] congener ratio ($P < 0.01$) and a more allergenic form of urushiol (29, 30).

Our findings indicate that under future levels of atmospheric CO_2, *T. radicans* may grow larger and become more noxious than it is today. Given the global distribution of this and other closely related species, these results have implications for forest dynamics and human health. Increased abundance of woody vines in

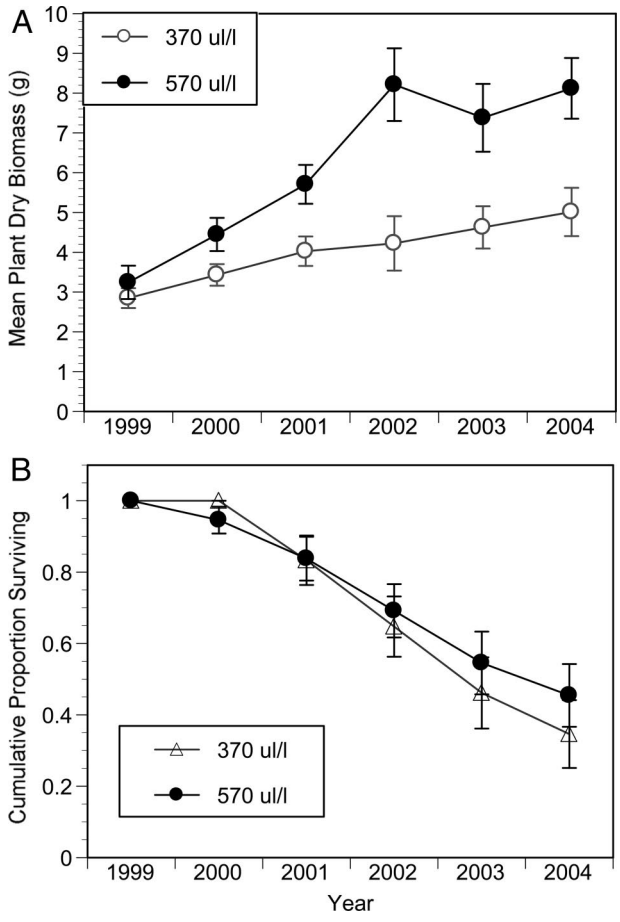

Fig. 1. Mean plant biomass and cumulative proportion surviving from 1999 to 2004. (*A*) Mean plant aboveground dry biomass calculated from a species- and site-specific allometric equation (see *Materials and Methods*). Error bars denote ± 1 SE. The rate of increase is greater at elevated CO_2 ($P = 0.022$ in a repeated-measures analysis; $n = 3$). (*B*) Mean cumulative proportion of stems surviving aboveground over time. Error bars denote ±1 SE. Kaplan–Meier survivorship analysis indicates no significant effect of CO_2.

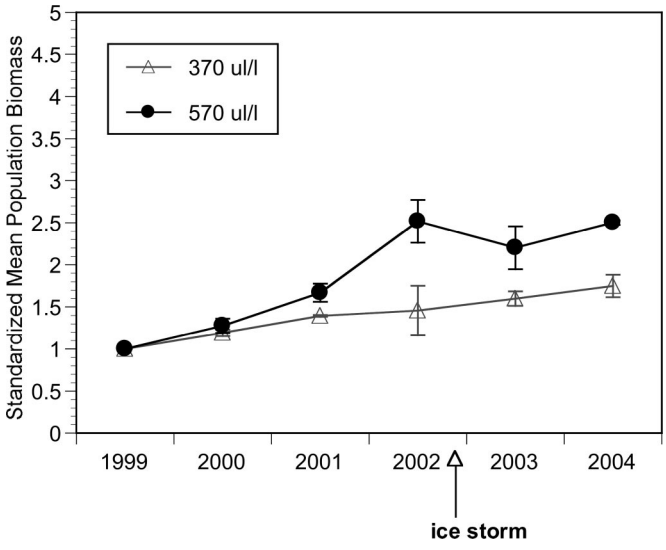

Fig. 2. Mean population biomass standardized by dividing by the initial plot biomass in 1999 ($n = 3$). Error bars denote ±1 SE. The rate of increase is greater at elevated CO_2 ($P = 0.046$ in a repeated-measures analysis).

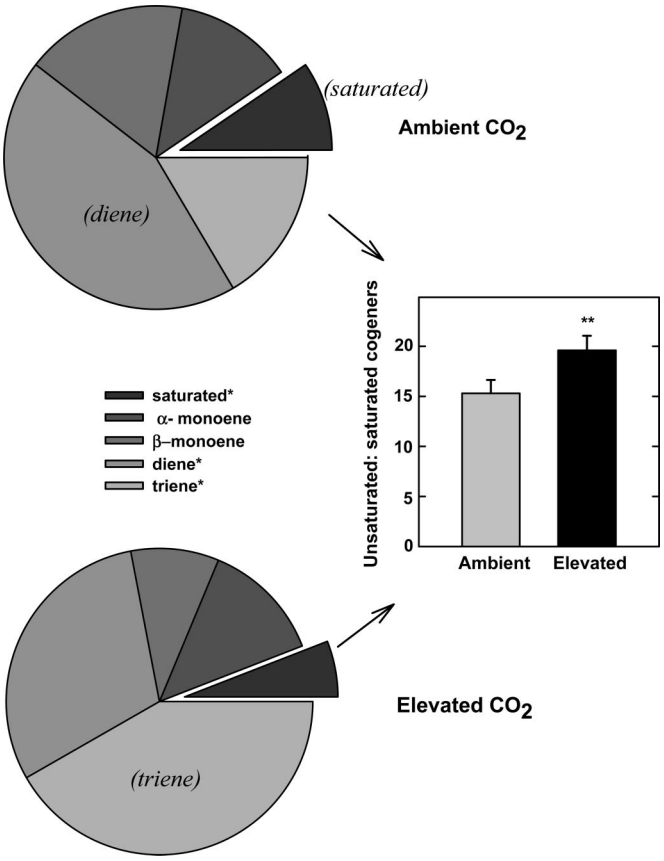

Fig. 3. Relative proportion of known congeners of urushiol in poison ivy sampled from May to September at the Duke FACE site in 2004. Under elevated CO_2, the concentration of the unsaturated triene congener of urushiol increased 153% ($P < 0.001$), whereas the saturated urushiol variant decreased by 61% ($P < 0.001$), resulting in an increased [unsaturated:saturated] congener ratio ($P < 0.01$). Bar graph represents the ratio of [unsaturated:saturated] congeners for urushiol extracted from leaves growing at ambient and elevated CO_2 concentrations. The higher the ratio of [unsaturated:saturated] variants, the more allergenic urushiol is to humans (29, 30). Error bars denote +1 SE.

old-growth and fragmented forests is reducing tree regeneration and increasing tree mortality in tropical (18, 19, 22, 23) and temperate (20, 21) regions. Our results support the proposal that elevated atmospheric CO_2 is at least partially responsible for this increased abundance in forested ecosystems (19, 23). In terms of human health, poison ivy contact dermatitis is an allergic response, in which symptoms often are exacerbated over time with increasing exposure to urushiol (27). If *Toxicodendron* becomes both more abundant and more irritating to sensitive individuals, which include ≈80% of the human population (33), it is likely that this plant will become a greater health problem in the future. Other species in the Anacardiaceae family, including mango, cashew, and pistachio, also can be allergenic (3). It is possible that these plants, too, may become more problematic in the future. The fertilization effect of rising CO_2 on poison ivy photosynthesis, biomass, and the shift toward a more allergenic form of urushiol have important implications for the future health of both humans and forests.

Materials and Methods

Site Description. CO_2 treatments commenced in September 1996 in three ambient (≈370 μl/liter) and three elevated (+200 μl/liter) plots, each 707 m^2 in area, in an unmanaged loblolly pine (*Pinus taeda*, L.) plantation at the Duke Forest FACE site (34). Infertile Ultic Alfisol soils occur at this site in the Piedmont of North Carolina (35°97′N 79°09′W). During the course of this study, mean global CO_2 rose from <365 to ≈375 μl/liter, but for simplicity ,all ambient CO_2 levels in this paper are denoted by 370 μl/liter and elevated concentrations by 570 μl/liter.

Physiology. Light-saturated net photosynthetic rates and stomatal conductance to water were measured at the growth CO_2 concentration (370 or 570 μl/liter CO_2) on two fully expanded shade leaves per plot in July 1999 by using an open-flow gas-exchange system (LI6400; Li-Cor, Lincoln, NE). Photosynthetic light-response curves were used to estimate apparent quantum yield of CO_2 fixation, light-compensation point, and light-saturation point. Photosynthetic CO_2–response curves were used to estimate light-saturated rates of carboxylation and ribulose bisphosphate regeneration mediated by electron transport (35). For all analyses, plot means are used as replicates.

Growth, Survivorship, and Population Biomass. In September 1999, the heights and diameters at 5-cm height (at permanently marked locations on stems) were recorded from 63 randomly chosen poison ivy plants growing in the FACE understory, with an average of 11 ± 1 individuals per plot. Plants in this system are browsed by white-tailed deer (*Odocoileus virginianus*), and poison ivy is typically <70 cm tall. To minimize the destructive impact of deer on the understory vegetation, we surrounded each plant with an herbivore exclosure constructed from 4-cm plastic mesh. Per-plant aboveground dry biomass was calculated from an allometric equation relating $\log_e$(biomass) to $\log_e$(plant height) and $\log_e$(stem diameter) that was generated from similarly sized plants harvested in the adjacent forest ($R^2 = 0.74$; $P < 0.0001$). A previous metaanalysis (31) and data from other woody understory species at this site suggest CO_2 does not alter plant allometric relationships. Plant size and aboveground survivorship were censused annually through September 2004. Total population biomass was calculated by multiplying the average plant biomass by the mean plant density, per plot, to get population biomass in terms of $g \cdot m^{-2}$. We standardized all population biomass data by dividing the total biomass in each plot per year by the initial 1999 biomass for that plot and then averaged the values to obtain the mean standardized biomass per CO_2 concentration per year. The rates of growth in plant ($g \cdot year^{-1}$) and population ($g \cdot m^{-2} \cdot year^{-1}$) biomass were analyzed by using repeated-measures ANOVA. Plots were used as replicates ($n = 3$). CO_2 impacts on aboveground stem survivorship were assessed by using a Kaplan–Meier survivorship analysis, but we did not detect a significant effect of CO_2.

Urushiol Analyses. An average of five leaves were harvested monthly from each plot between May and September 2004. Leaf samples were transferred to the laboratory on dry ice and were stored at −80°C before analysis. Leaves were collected in the understory and occasionally from tall vines growing into the canopy. There were no statistically significant trends in leaf urushiol content with plant height or on a diurnal basis.

Frozen poison ivy leaflets (0.1 to 0.2 g fresh weight) were ground to a fine powder with a mortar and pestle, and the tissue was extracted three times with 2 ml of ethanol (36). The combined extracts were centrifuged for 15 min at 8,000 × *g* in a Jouan model MR23i centrifuge, and the supernatants were partitioned with $CHCl_3$ and H_2O (1:1). The organic fraction was evaporated to dryness under N_2 at 37°C, and the samples were resuspended in 1 ml of 95% ethanol. A 50-μl aliquot of each sample was dried overnight in a vacuum desiccator and was derivatized for 30 min at 37°C with 0.1 ml of *N*-methyl-*N*-(trimethylsilyl)fluoracetamide. Derivatized urushiols were separated by gas chromatography (model 6890A; Hewlett–Packard) by using methods similar to those previously used for *Toxico-*

dendron (36, 37), and urushiol was detected with a mass selective detector coupled to CHEMSTATION (Hewlett–Packard). Separations were performed with a 30-m x 0.25-mm SPB-50 column (Supelco) by using high-purity helium as a carrier gas at 1.2 $ml \cdot min^{-1}$. The oven temperature was increased from 150°C to 275°C at $5°C \cdot min^{-1}$. Up to five individual urushiol congeners [the saturated form (m/e 464), two forms of the monoene (m/e 462), the diene (m/e 460), and the triene (m/e 458)] were detected in poison ivy leaf extracts, and trimethylsilyl derivatives were identified by the presence of a base peak at m/e 179. Quantitation was based on standard curves prepared with known concentrations of urushiols from a partially purified poison oak (*Toxicodendron diversilobum*) sample and standard recoveries by using 3-pentadecylphenol were >90%. Analysis of variance was used to examine effects of CO_2 ($\mu l \cdot liter^{-1}$) on a relative fraction of urushiol congeners and urushiol concentration from poison ivy (*Toxicodendron radicans*) foliage.

We thank P. Frankson, D. LeBauer, J. Pippen, J. McKellips, and M. Anderson for assistance in the field; M. El Sohly (University of Mississippi, University, MS) for contributing the poison oak standard used in urushiol analyses; E. Goins and D. Reed for their efforts in the laboratory and the field; D. Myers for providing valuable assistance with the gas exchange measurements; and P. Frankson, F. Putz, D. Pimental, R. Hanifin, and B. Von Holle for providing valuable comments on the manuscript. This research was funded in part by the U.S. Department of Energy and the National Science Foundation.

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